P ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 November 2000 (22.11.00)	
International application No. PCT/GB00/01079	**Applicant's or agent's file reference** JEC/BP5846738
International filing date (day/month/year) 22 March 2000 (22.03.00)	**Priority date** (day/month/year) 22 March 1999 (22.03.99)
Applicant PELICCI, Pier, Giuseppe et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 October 2000 (16.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Zakaria EL KHODARY

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
JEC/BP5846738		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 01079	22/03/2000	22/03/1999
Applicant		
CANCER RESEARCH VENTURES LIMITED		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 4

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Please delete lines 1-6, Text "the invention concerns..." to "and prolongs survival."

Abstract now begins at "It has been determined..."

INTERNATIONAL SEARCH REPORT

International Application No
GB 00/01079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/11 C07K14/47 C12Q1/68 G01N33/53
A61K39/395 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARUN R B ET AL: "Characterization of human SHC p66 cDNA and its processed pseudogene mapping to Xq12-q13.1" GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 42, no. 2, 1 June 1997 (1997-06-01), pages 349-352-352, XP002107843 ISSN: 0888-7543	1,2,5-8, 39,42,44
A	page 349, column 2 -page 352, column 1; figure 2 -& HARUN ET AL.: "shc transforming protein" EMBL DATABASE ACC. NO: Y09847, 1 December 1992 (1992-12-01), XP002142438 abstract	3,4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2000	26/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 17866 A (UNIV CALIFORNIA) 13 June 1996 (1996-06-13) page 27, line 16 -page 38, line 12; claims 1-34; figures 1,2,5	32,35, 39-44
X	EL-SHEMERLY ET AL: "12-O-Tetradecanoylphorbol-13-acetate activates the Ras/extracellular signal-regulated kinase (ERK) signalling pathway upstream of SOS involving serine phosphorylation of Shc in NIH3T3 cells" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 49, 5 December 1997 (1997-12-05), pages 30599-30602, XP002142439 page 30601, column 1 figures 1-3	32-35, 41,43
X	LESLIE NICK R ET AL: "An activating mutation in the kit receptor abolishes the stroma requirement for growth of ELM erythroleukemia cells, but does not prevent their differentiation in response to erythropoietin." BLOOD, vol. 92, no. 12, 15 December 1998 (1998-12-15), pages 4798-4807, XP000915258 ISSN: 0006-4971	32-35
A	page 4800, column 1 page 4803; figure 6	12,19, 36,43
A	MIGLIACCIO ET AL.: "Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway" THE EMBO JOURNAL, vol. 16, no. 4, 1997, pages 706-716, XP002142441 page 711, column 2; figures 1-9	1-44
A	RAO ET AL.: "Role of hydroperoxyeicosatetranoic acids in oxidative stress-induced activating protein 1 (AP-1) activity" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1 November 1996 (1996-11-01), pages 27760-27764, XP002142442 page 27760 figure 2	1-44

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5

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MIGLIACCIO ET AL.: "The p66shc adaptor protein controls oxidative stress response and life span in mamals" NATURE, vol. 402, 18 November 1999 (1999-11-18), pages 309-313, XP002142443 the whole document	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/GB 00/01079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9617866 A	13-06-1996	US 5744313 A	28-04-1998
		AU 4367196 A	26-06-1996
		EP 0871661 A	21-10-1998
		JP 10510422 T	13-10-1998
		US 5925547 A	20-07-1999

14

TENT COOPERATION TR Y

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
JEC/BP5846738		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01079	22/03/2000	22/03/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant CANCER RESEARCH VENTURES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/10/2000	19.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Morawetz, R Telephone No. +49 89 2399 8155

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01079

I. Basis of the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-3,5-32,34-47	as originally filed			
4,33	as received on	04/08/2000	with letter of	01/08/2000

Claims, No.:

1-44	as originally filed

Drawings, sheets:

1/14-14/14	as originally filed

Sequence listing part of the description, pages:

1-5, filed with the letter of 13.06.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-21, 28-31 and 36-38.

because:

☒ the said international application, or the said claims Nos. 9-21 and 36-38 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear

that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 28-31 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 4, 6-8, 12-27, 36-38
	No:	Claims	1, 2, 5, 9-11, 32-35, 39-44
Inventive step (IS)	Yes:	Claims	3 (partially, i.e. insofar restricted to S36), 4, 12-27, 36-38
	No:	Claims	3 (partially, i.e. insofar related to S28 or S54) and 6-8
Industrial applicability (IA)	Yes:	Claims	1-8, 22-27, 32-35, 39-44
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I
Basis of the report

1. The amendments filed with the letter dated 1.8.2000 are considered allowable under Article 34(2)(b) PCT.

   The amendments concern the deletion of passages (description, page 4, lines 9-11 and lines 21-23; page 33, lines 25-27) relating to statements that the targeted mutation of the mouse $p66^{shc}$ gene increases susceptibility to tumour and that $p66shc^{-/-}$ mice are more susceptible to chemically-induced carcinogenesis. The applicant argues that these statements should be deleted because they are incorrect.

2. This authority is of the opinion that the passage on page 22, line 1-8 should also be deleted, because it relates to the same incorrect statement.

Re Item II
Priority

1. The document D7 (MIGLIACCIO ET AL., NATURE, vol. 402, 18 November 1999 (1999-11-18), pages 309-313) indicated in the search report as a P-document is not to be regarded as state of the art according to Article 33(2) PCT for the present set of claims, as the date of priority claimed can be allowed for the relevant parts of the present application.

Re Item III
Non-establishment of report with regard to novelty, inventive step or industrial applicability

1. Claims 28-31 are so inadequately supported by the description (Article 6 PCT) that no meaningful examination regarding novelty, inventive step or industrial applicability is possible.
   The applicant argues in his letter dated 1.8.2000 that deletion of the incorrect

statements (see above, item I) has no bearing on the claims.

This authority respectfully disagrees with this point of view. Claims 28-31 are clearly based on the assumption that increasing the expression of $p66^{shc}$ would lead to an increased resistance to tumour, because $p66shc^{-/-}$ mice are more susceptible to chemically-induced carcinogenesis (which they apparently are not). Given that the incorrect statements have now been deleted from the description and since no other basis for these claims could be identified, this authority is, consequently, of the opinion that claims 28-31 are inadequately supported by the description (Article 6 PCT).

2. Claims 9-21 and 36-38 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents, the numbering corresponds to the listing of the documents in the international search report:

D1: HARUN R B ET AL., GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 42, no. 2, 1 June 1997 (1997-06-01), pages 349-352-352, & HARUN ET AL., EMBL DATABASE ACC. NO: Y09847, 1 December 1992 (1992-12-01)

D3: EL-SHEMERLY ET AL., THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 49, 5 December 1997 (1997-12-05), pages 30599-30602

D4: LESLIE NICK R ET AL., BLOOD, vol. 92, no. 12, 15 December 1998 (1998-12-15), pages 4798-4807

D5: MIGLIACCIO ET AL., THE EMBO JOURNAL, vol. 16, no. 4, 1997, pages 706-716

D6: RAO ET AL., THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1 November 1996 (1996-11-01), pages 27760-27764

2. The present application does not satisfy the criterion set forth in **Articl 33(2) PCT** because the subject-matter of claims 1, 2, 5, 9-11, 32-35, 39-44 is not new in respect of prior art as defined in the regulations (**Rule 64(1)-(3) PCT**).

2.1. Present application provides methods and materials relating to the observation that $p66^{shc}$ is involved in the signal transduction pathway that regulates stress response and lifespan in mammals.
The application discloses that $p66^{shc}$ is phosphorylated at serine 36 upon UV treatment or oxidative damage; that ablation of $p66^{shc}$ expression by homologous recombination enhances resistance to oxidative damage both in vitro and in vivo and that $p66^{shc\ -/-}$ mice have a prolonged lifespan.

2.2. The subject-matter of claims 1, 2, 5, 39, 40 and 42 is considered anticipated by D1.

D1 discloses a human pseudogene (Pseudo SHC p66 cDNA) encoding human Pseudo SHC66 which has several serine residues (S80, S213, S335, S476) replaced by a different amino acid, when compared to the wild type sequence or the sequence shown in Fig. 5 and, thus, anticipates the subject-matter of claims 1, 2 and 5.
The human SHC66 sequence of D1 also differs from the sequence of Fig. 5 in that the serine at position 38 is replaced by a proline.
The mouse shc66 of D1 has also several serine residues replaced by a different amino acid (S60, S80, S102) when compared to the wild type sequence or the sequence shown in Fig. 5.
D1 furthermore discloses the use of oligonucleotides according to claims 39 and 40 and a method according to claim 42.

2.3. The subject-matter of claims 32-35, 41 and 43 is considered anticipated by D3.

D3 discloses (page 30600, right hand column, last paragraph - page 30602, left hand column, last paragraph) methods which fall within the scope of claims 32-35.
D3 also discloses (e.g. Figures 3, 4) the use of an antibody according to claim 41 and a method according to claim 43.

2.4. The subject-matter of claims 9-11, 32-35, 39-44 is considered anticipated by D5.

D5 (by the inventors) discloses the sequence of human $p66^{shc}$ (GenBank accession number U73377) which is identical with the sequence shown in Figure 5 of present application. D5 also discloses subcloning of $p66^{shc}$ into the pMT2 vector for transient expression in COS-1 and HeLa cells, the generation and use of antibodies against $p66^{shc}$, that $p66^{shc}$ is tyrosine-phosphorylated upon epidermal growth factor stimulation and that $p66^{shc}$ inhibits fos promoter activation.

2.5. The subject-matter of claims 3, 4, 6-8, 12-27, 36-38 appears to be novel in view of the available prior art.

3. The present application does not satisfy the criterion set forth in **Article 33(3) PCT** because the subject-matter of claims 3 (partially, i.e. insofar related to S28 or S54) and 6-8 does not involve an inventive step as defined in the regulations (**Rule 65 (1)-(2) PCT**).

3.1. The subject-matter of claim 3 (partially, i.e. insofar related to S28 or S54) does not appear to solve any technical problem and is, thus, considered to lack an inventive step. Claims 6-8 concern embodiments which are familiar to the skilled person. Consequently, they would only be considered inventive if they were based upon a new and inventive nucleic acid molecule. For the present claims 6-8 this is not the case. Therefore the subject-matter of these claims is also considered to be obvious.

4. Claims 3 (partially, i.e. insofar restricted to S36), 4, 12-27, 36-38 are considered to fulfil the criteria of Article 33(2) and (3) PCT since, in the light of the available prior art, they define what appears to be new and inventive subject-matter, namely methods and materials relating to the observation that $p66^{shc}$ is phosphorylated at serine 36 upon UV treatment or oxidative damage and that it is involved in the signal transduction pathway that regulates stress response and lifespan in mammals.

$P66^{shc}$ and its involvement in known signal transduction pathways (e.g. growth factor transduction pathways) and the negative regulation of c-fos promoter

activity were known from the prior art (see D5). It was also known (see D6) that hydrogen peroxide induces c-Fos expression. This authority is of the opinion that in view of the teaching of D5 in combination with D6 the skilled person had, however, no reasonable expectation that $p66^{shc}$ modulates the oxidative stress response or that serine phosphorylation at serine 36 of $p66^{shc}$ is necessary for a normal stress response.

Re Item VIII

Certain observations on the international application

1. Article 6 PCT and Rule 6 PCT

1.1. Claims 12-22 are not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description and drawings. The reasons therefor are the following: it is clear from the description (page 31, lines 27-32 and page 43, line 15-20) that $p66^{shc}$ is involved in the intracellular transduction pathways of both environmental stresses and growth factors. UV and H_2O_2 induce rapid and persistent serine-phosphorylation at serine 36, while EGF induced rapid an transient tyrosine-phosphorylation. Consequently, only claims restricted to the disruption of the environmental stress-related pathway of $p66^{shc}$ and phosphorylation of $p66^{shc}$ at serine 36 are considered supported by the description as required by Article 6 PCT.

phosphorylation of p66 by oxidative signals is mediated by Erk1 and p38, as shown both in vivo and in vitro; iii) ablation of $p66^{shc}$ expression by homologous recombination enhances resistance to oxidative damage both *in vitro* and *in vivo*; iv) a serine-phosphorylation defective mutant of $p66^{shc}$ is unable to restore a normal stress response in $p66^{shc}$ targeted cells; v) mice carrying the $p66^{shc}$ targeted mutation have prolonged lifespan.

Furthermore, the present inventors have determined that targeted mutation of the mouse $p66^{shc}$ gene increases susceptibility to tumour formation. The present inventors disclose herein that i) p16, p53 and p21 activation is lost in p66-/- cells upon H_2O_2 or UV treatment or RASV12 expression; ii) the oncogenic RASV12 is unable to induce cell senescence into p66-/- mouse embryo fibroblasts (MEFs) and, on the contrary, it transforms p66-/- cells; iii) p66-/- MEFs over-expressing RASV12 show a transformed, spindle-shaped morphology, are capable of forming foci at confluency and colonies in semisolid media; iv) p16 and p53 are unable to induce growth proliferation of p66-/- cells; v) p66-/- mice are more susceptible to chemically-induced carcinogenesis than littermates.

Thus, the present inventors show herein that p66 itself is activated by serine phosphorylation by stress activated kinases and signals to p16-p19-p53-p21 and that functionally, the p66 signalling pathway regulates tumour supression and lifespan.

Therefore, at its most general, the present invention provides materials and methods associated with the modulation of $p66^{shc}$ gene expression and its involvement in a signal transduction pathway that is activated by environmental stresses and oncogenic

death induced by H_2O_2; ii) p66-/- MEFs are more resistant to H_2O_2-induced cell death than wild-type controls *in vivo*. Paraquat is a pesticide that kills mice by inducing oxidative damage. The present inventors have further demonstrated that p66-/- mice are more resistant to paraquat treatment than littermates.

7) p66 regulates the p16, p53 and p21 response

Since environmental stresses activates the p16 - p53-p21 signalling pathways, the present inventors have further investigated whether p66 interferes with p16-p53-p21 activation by H_2O_2. Results revealed that p16, p53 and p21 activation are lost in p66-/- cell upon H_2O_2 treatment.

8) p66 is a tumour supressor

In vitro, the stimulatory effect of p66 on the p53-p21 pathway suggests that it might play a role in the cellular response on oncogenic stimuli. Therefore, the present inventors have evaluated the effects of p66 on the response of primary fibroblasts on the oncogenic RASV12 mutant. RASV12 induces senescence of wild-type MEFs, as a consequence of p53-p21 activation. Expression of RASV12 into p66-/- MEFs induced cellular transformation. *In vivo*, p66-/- mice are more susceptible to chemical-induced carcinogenesis than littermates. Furthermore, the present inventors have demonstrated that p53 and p16 are unable to induce senescence of mouse p66-/- fibroblasts.

9) p66 mediates aging

The results presented herein demonstrate that p66 is involved in the cellular response to stresses

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/01079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/11 C07K14/47 C12Q1/68 G01N33/53
A61K39/395 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARUN R B ET AL: "Characterization of human SHC p66 cDNA and its processed pseudogene mapping to Xq12-q13.1" GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 42, no. 2, 1 June 1997 (1997-06-01), pages 349-352-352, XP002107843 ISSN: 0888-7543	1,2,5-8, 39,42,44
A	page 349, column 2 -page 352, column 1; figure 2 -& HARUN ET AL.: "shc transforming protein" EMBL DATABASE ACC. NO: Y09847, 1 December 1992 (1992-12-01), XP002142438 abstract	3,4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2000	26/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 17866 A (UNIV CALIFORNIA) 13 June 1996 (1996-06-13) page 27, line 16 -page 38, line 12; claims 1-34; figures 1,2,5	32,35, 39-44
X	EL-SHEMERLY ET AL: "12-O-Tetradecanoylphorbol-13-acetate activates the Ras/extracellular signal-regulated kinase (ERK) signalling pathway upstream of SOS involving serine phosphorylation of Shc in NIH3T3 cells" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 49, 5 December 1997 (1997-12-05), pages 30599-30602, XP002142439 page 30601, column 1 figures 1-3	32-35, 41,43
X	LESLIE NICK R ET AL: "An activating mutation in the kit receptor abolishes the stroma requirement for growth of ELM erythroleukemia cells, but does not prevent their differentiation in response to erythropoietin." BLOOD, vol. 92, no. 12, 15 December 1998 (1998-12-15), pages 4798-4807, XP000915258 ISSN: 0006-4971	32-35
A	page 4800, column 1 page 4803; figure 6	12,19, 36,43
A	MIGLIACCIO ET AL.: "Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway" THE EMBO JOURNAL, vol. 16, no. 4, 1997, pages 706-716, XP002142441 page 711, column 2; figures 1-9	1-44
A	RAO ET AL.: "Role of hydroperoxyeicosatetranoic acids in oxidative stress-induced activating protein 1 (AP-1) activity" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1 November 1996 (1996-11-01), pages 27760-27764, XP002142442 page 27760 figure 2	1-44

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	MIGLIACCIO ET AL.: "The p66shc adaptor protein controls oxidative stress response and life span in mamals" NATURE, vol. 402, 18 November 1999 (1999-11-18), pages 309-313, XP002142443 the whole document	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[redacted]l Application No
PCT/GB 00/01079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9617866 A	13-06-1996	US 5744313 A	28-04-1998
		AU 4367196 A	26-06-1996
		EP 0871661 A	21-10-1998
		JP 10510422 T	13-10-1998
		US 5925547 A	20-07-1999

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
CRIPPS, Joanna, E.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

Date of mailing (day/month/year) 28 September 2000 (28.09.00)	
Applicant's or agent's file reference JEC/BP5846738	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/GB00/01079	22 March 2000 (22.03.00)	22 March 1999 (22.03.99)

Applicant
CANCER RESEARCH VENTURES LIMITED et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   28 September 2000 (28.09.00) under No. WO 00/56886

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
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